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Prospective evaluation of a dot-blot enzyme immunoassay (Directigen RSV) for the antigenic detection of respiratory syncytial virus from nasopharyngeal aspirates of paediatric patients

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ABSTRACT

This study investigated the efficacy of a commercial enzyme immunoassay (Directigen RSV, ColorPAC) in comparison with the shell vial culture method (using Hep-2 cells) for the detection of respiratory syncytial virus (RSV) in nasopharyngeal aspirates from children with bronchiolitis. During the period 1995–2002, 4950 samples were examined. RSV was detected in 1660 (33.5%) samples, with a sensitivity of 80.9%, a specificity of 97.5%, a positive predictive value of 93.8%, a negative predictive value of 91.6%, and a testing efficiency value of 92.2% compared with shell vial culture. In 83 (5%) samples, the ColorPAC was positive and the shell vial assay was negative. Of these, 71 (85.6%) were false-negative by cell culture. The true false-positive results obtained by ColorPAC represented only 0.7% of all RSV-positive samples. In general, no statistically significant differences were detected between the different months and epidemic periods studied. Compared with ColorPAC, the shell vial culture method displayed a sensitivity of 95.8% and a specificity of 100%. Overall, the ColorPAC assay was an acceptable, simple and rapid method for the antigenic detection of RSV in paediatric respiratory samples.

Keywords ColorPAC, enzyme immunoassay, nasopharyngeal aspirates, respiratory samples, respiratory syncytial virus

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INTRODUCTION

Respiratory syncytial virus (RSV) is recognised as the principal causative agent of acute lower respiratory tract infections affecting infants and young children aged <2 years. Most infections occur during annual epidemic outbreaks in winter and early spring. In the hospital setting, RSV is transmitted efficiently from patient to patient in the absence of appropriate infection control measures [1,2]. Therefore, it is important to reach an early diagnosis that permits the rapid identification of infected children [3,4].

Two rapid techniques are available for the diagnosis of infection by RSV, based on either virus antigen detection in respiratory secretions (enzyme immunoassay) or direct immunofluorescence [5]. Virus isolation in cell culture is considered to be the standard against which all new techniques for detection and isolation of RSV should be compared [6,7]. However, the shell vial culture method has been shown to be more sensitive and rapid than classic cell culture, suggesting that this method may substitute for culture in the study of epidemic outbreaks of bronchiolitis caused by RSV [8,9]. This article describes a prospective study of the efficacy of a rapid antigen detection method, in comparison with the shell vial method of isolation in culture, for the detection of RSV in nasopharyngeal aspirates from children with a clinical diagnosis of bronchiolitis.

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MATERIALS AND METHODS

This was a prospective study that included all paediatric patients (aged <14 years) who attended the emergency department of University Hospital Son Dureta (Palma de

Mallorca, Spain) in the November–March period for seven consecutive years (1995–2002). Nasopharyngeal aspirates from these patients were inoculated in liquid transport medium (Earle's minimum essential medium (MEM) with bovine serum albumin 0.5% w/v) and sent as soon as possible to the virology laboratory. On arrival at the laboratory, the nasopharyngeal samples were diluted in 3 mL of sterile saline solution. The rapid antigen detection method, which involved an enzyme immunomembrane filter assay (ColorPAC, Directigen RSV; Becton Dickinson, Franklin Lakes, NJ, USA) was performed with 250 μL of the sample, following the manufacturer's instructions both for the preparation of the sample and for reading the results. Samples giving indeterminate results were retested according to the manufacturer's instructions.

For the shell vial technique, 200 μL of the same sample was inoculated into two Hep-2 vials (Vircell; Ingelheim Diagnostica, Madrid, Spain), centrifuged at 700 *g* for 45 min, and incubated at 36°C for 60 min; after this, the supernatant was discarded. One millilitre of maintenance medium (MEM containing fetal bovine serum 1% v/v) was added to each sample, and the vials were incubated at 36°C for 2 days. After incubation, the monolayers were stained with anti-RSV (Monofluokit RSV; Bio-Rad, Madrid, Spain) and viewed at ×200 and ×400 magnification with a fluorescence microscope.

Enzyme immunoassay-positive, culture-negative samples (stored at 4°C) were re-inoculated into vials of Hep-2, MDCK, LLC-MK2 and MRC-5 cell lines and incubated for 3 days at 36°C, after which the monolayers were stained with anti-RSV, anti-adenovirus, anti-influenza virus, anti-parainfluenza 1, 2 and 3 (Monofluokit; Bio-Rad) and anti-enterovirus (Dako, Ely, UK) monoclonal antibodies.

For statistical analysis, all positive cultures were considered to be true positives. Samples that were ColorPAC-positive, but from which viruses other than RSV were isolated, were considered to be probable false-positives. Samples that were ColorPAC-positive and culture-negative for other viruses were submitted to a direct immunofluorescence assay (DFA) against RSV (Monofluokit RSV; Bio-Rad). Negative shell vial cultures were considered to be false-negative if they were ColorPAC-positive, culture-negative and DFA-negative.

Sensitivity, specificity and positive and negative predictive values for the ColorPAC assay were calculated by comparison with isolation of RSV by shell vial culture. Testing efficiency was defined as the total number of correctly assigned testing values (true positives and true negatives for each assay) divided by the number of specimens tested. Statistical analysis was carried out by performing Student's *t*-test on paired data. All p values were two-tailed and were considered significant if < 0.05 [10].

RESULTS

Only samples received during the months of greatest incidence of RSV infection (November–March), for seven consecutive years, were included in this prospective study. In total, 4950 samples were analysed, and 1660 (33.5%) were positive for RSV. The ColorPAC assay and shell vial culture were positive for 1277 (76.8%) samples; for 300 (18.1%) samples, only the shell vial culture was positive; and for 83 (4.9%) samples, only the ColorPAC assay was positive. Thus, comparing the ColorPAC assay with shell vial culture, the ColorPAC assay had a sensitivity of 80.9%, a specificity of 97.5%, a positive predictive value of 93.8%, a negative predictive value of 91.6%, and a testing efficiency value of 92.2%.

The 83 samples considered initially to be false-positive ColorPAC results represented 4.9% of the RSV-positive samples and 1.6% of all samples. Of these, 71 (85.6%) were ultimately considered to be shell vial culture false-negatives, because no other virus could be isolated and the DFA was positive. These samples represented 4.2% of the RSV-positive samples and 1.4% of all samples. With 12 (14.4%) samples, it was possible to isolate a virus after re-inoculating the shell vial cultures (nine influenza viruses (six influenza B and three influenza A), two adenovirus, and one parainfluenza virus). These samples were considered to be true ColorPAC false-positives, as the DFA was also negative, and represented 0.7% of RSV-positive samples and 0.25% of all samples.

In order to analyse the efficacy of the ColorPAC assay, the samples were first grouped cumulatively according to individual months (Table 1). The only significant differences were between the percentages for positive results ($p < 0.05$) and sensitivity (p 0.051) for RSV detection in the month of November and the average values detected in all the samples studied. Non-significant differences were detected in the values for specificity, positive predictive value, negative predictive value and testing efficiency value in the different months studied (Fig. 1).

In a second analysis, the clinical samples were grouped according to annual epidemic periods (Table 2). A statistical difference in the percentage of positive results, with respect to the overall percentages obtained, was observed only in the epidemic periods of 1995 and 1996 (Fig. 2). No significant differences were detected between the epidemic periods over the entire length of the study.

Compared with the ColorPAC assay, shell vial culture had a sensitivity of 95.8% and a specificity of 100%, since the ColorPAC assay gave true-positive results for 71 samples that yielded no growth in shell vial culture.

Table 1. Monthly results obtained during the prospective study period (1995–2002)

Month	Total	No. (%) RSV-positive	Test sensitivity (%)	Test specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Testing efficiency value
November	509	59 (11.5)	67.9	98.6	85.7	96.3	95.4
December	1077	438 (40.6)	78.1	98.1	96.5	87.2	90.2
January	1350	565 (41.8)	83.2	98.6	97.6	89.4	92.2
February	1179	412 (34.9)	81.4	96.2	91.2	91.5	91.4
March	835	186 (22.5)	83.9	96.4	85.0	96.1	94.0
Total	4950	1660 (33.5)	80.9	97.5	93.8	91.6	92.2

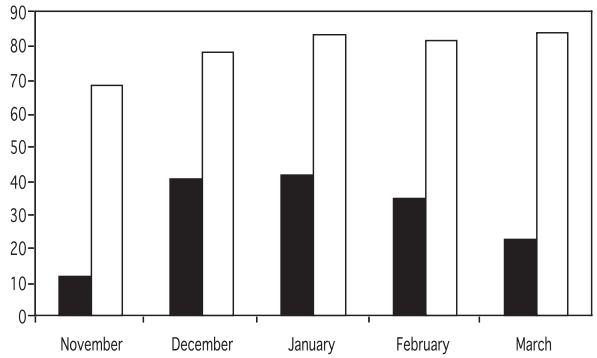

Fig. 1. Comparison between the percentage of RSV-positive samples (solid bars) and sensitivity (open bars) of the ColorPAC assay in each month during the epidemic periods.

DISCUSSION

The annual epidemics of bronchiolitis caused by RSV affect *c*. 10–20% of the population aged <2 years. Of this group, nearly 20% require hospitalisation because of the severity of the disease and the young age of the patients [1,2]. As RSV is easily disseminated and transmitted, it is essential to obtain a rapid aetiological diagnosis of this infection in order to allocate patients to appropriate hospital zones or isolation wards. The aim is to avoid an outbreak of RSV nosocomial infections, which, in general, have a more severe clinical outcome than community-acquired infections [3,4].

Of the different rapid diagnostic methods available for RSV, enzyme immunoassay systems provide high sensitivity and specificity, as well as being appropriate for routine use, with results available in <15 min [5,10,11]. The present prospective study of the ColorPAC assay demonstrated acceptable levels of sensitivity (80.9%) and specificity (97.5%). Only samples received during the months considered to have the highest rate of RSV infections (November–March) were included in the study, since the screening methods used could display significant variations in efficacy according to the prevalence of the infection under consideration [11–13]. Overall, the percentages observed were higher than those obtained (sensitivity 62.5% and specificity 91.8%) in a previous study, carried out with a lower number of samples in the period 1995–1996 [14]. They were also higher than those reported by some other research groups, ranging from 57% [15] to 76% [16], although some authors have reported sensitivity percentages close to 98% [17–20].

The ColorPAC assay was positive and the shell vial culture was negative in 83 (5%) samples; these were initially considered to be probable false-positives of the antigen detection system. This percentage was lower than that observed (7.7%) in our previous study [14], but was similar to that reported (4.8%) by Ribes *et al.* [20]. On re-inoculation of these samples, a virus other than RSV was isolated in 12 (14.4%) cases. Therefore, only these samples should be considered as

Table 2. Overall results obtained in the prospective study period (1995–2002)

Period	Total	No. (%) RSV-positive	Test sensitivity (%)	Test specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Testing efficiency value (%)
1995–1996	373	184 (49.3)	82.3	98.4	98.0	85.5	90.6
1996–1997	554	224 (40.6)	82.0	94.8	90.3	89.8	89.8
1997–1998	504	170 (33.7)	81.5	99.4	98.5	91.5	93.4
1998–1999	596	219 (36.7)	78.4	96.1	91.4	89.5	90.1
1999–2000	1027	317 (30.8)	83.1	96.4	90.2	93.5	92.6
2000–2001	982	346 (35.2)	77.8	97.3	93.7	89.7	90.8
2001–2002	914	200 (21.8)	82.8	99.7	98.7	95.4	96.1
Total	4950	1660 (33.5)	80.9	97.5	93.8	91.6	92.2

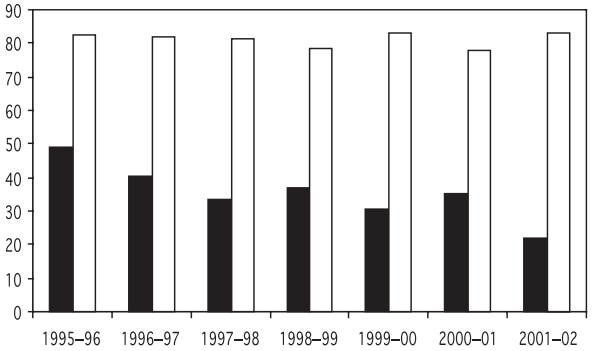

Fig. 2. Comparison between the percentage of RSV-positive samples (solid bars) and sensitivity (open bars) of the ColorPAC assay in the different annual epidemic periods.

probable false-positives, because no RSV was isolated following re-inoculation. Thus, the ColorPAC method was considered to yield probable false-positives with only 0.7% of all samples positive for RSV.

With 71 ColorPAC-positive samples, it was not possible to isolate any virus following re-inoculation, but the DFA was also positive. For these samples, it is possible that a delay in inoculation or instability of RSV prevented its growth in cell culture [8,10,14]. The comparison of the shell vial culture with the ColorPAC assay gave the latter a sensitivity of 95.8%, which is far higher than the values reported previously of 73–89% [8,13,14,18]. However, these percentages depend on the population (age and social status) and season of the year studied (months with higher prevalence), as well as the prevalence (rate) of infection during the period studied [1,2].

Because of the variability that these conditions impose on the results of antigen detection methods for most virus respiratory infections [1,2], the present study analysed the efficacy of ColorPAC in each of the epidemic months and in each epidemic period separately. The percentage of positive RSV samples varied according the month studied. The highest percentages (40.6% and 41.8%) were found in the classic epidemic months (December and January). Only the month of November had a positivity percentage lower than the average (33.5%) for the 5 months studied. Despite this difference, only a small, non-signficant difference was observed in the sensitivity of the ColorPAC assay for the month with the lowest rate of infection (i.e., November). When these data are combined, it can be seen that the ColorPAC assay had a stable, homogeneous efficacy, and did not appear to be excessively influenced by the rate of infection observed individually in each of the months studied.

The efficacy analysis of the ColorPAC assay in each of the epidemic periods showed a significant difference in the positivity percentage (49.3%) for the period 1995–1996 compared to the average (33.5%) obtained over the entire study period. Nevertheless, hardly any difference was observed in the different parameters that indicated the true efficacy of the ColorPAC assay, either in this or the following periods. Thus, the ColorPAC assay displayed statistical stability over the seven epidemic periods, with positivity percentages oscillating between 21.8% and 49.3%.

In summary, the commercially available ColorPAC assay had sensitivity and specificity percentages which were stable and independent of the annual rate of infection by RSV. It therefore seems that this assay is an acceptable, simple and rapid method for use in the antigenic detection of RSV, especially during the winter epidemic months.

REFERENCES

1. Collins PL, Chanock RM, Murphy BR. Respiratory syncytial virus. In: Knipe DM, Howley PM, eds. *Fields virology*, 4th edn. Philadelphia: Lippincott Williams & Wilkins, 2001; 1443–1486.
2. Hall CB. Respiratory syncytial virus. In: Feigin R, Cherry J, eds. *Textbook of pediatric infectious diseases*, 4th edn. Philadelphia: Saunders, 1998; 2084–2111.
3. Adcock PM, Stout GG, Hauck MA, Marshall GS. Effect of rapid diagnosis on the management of children hospitalized with lower respiratory tract infection. *Pediatr Infect Dis J* 1997; **16**: 842–846.
4. Karanfil LV, Colon M, Lykens K *et al*. Reducing the rate of nosocomially transmitted respiratory syncytial viral infections. *Am J Infect Control* 1999; **27**: 91–96.
5. Michaels MG, Serdy C, Barbadora K, Green M, Apalsch A, Wald ER. Respiratory syncytial virus: a comparison of diagnostic modalities. *Pediatr Infect Dis J* 1992; **11**: 613–616.
6. Subbarao EK, Whitehurst NJ, Waner JL. Comparison of two enzyme-linked immunosorbent assay (EIA) kits with immunofluorescence and isolation in cell culture for detection of respiratory syncytial virus (RSV). *Diagn Microbiol Infect Dis* 1987; **8**: 229–234.
7. Blanding JG, Hoshiko MG, Stutmam HR. Routine viral culture for pediatric respiratory specimens, submitted for direct immunofluorescence testing. *J Clin Microbiol* 1989; **27**: 1438–1440.
8. Smith MC, Creutz C, Huang YT. Detection of respiratory syncytial virus in nasopharyngeal secretions by shell vial technique. *J Clin Microbiol* 1991; **29**: 463–465.
9. Rabalais GP, Stout GG, Ladd KL, Cost KM. Rapid diagnosis of respiratory viral infections by using a shell vial

assay and monoclonal antibody pool. *J Clin Microbiol* 1992; **30**: 1505–1508.
10. Waner JL, Whitchurst NJ, Todd SJ, Shalaby H, Wall LV. Comparison of Directigen RSV with viral isolation and direct immunofluorescence for the identification of respiratory syncytial virus. *J Clin Microbiol* 1990; **28**: 480–483.
11. Halstead DC, Todd S, Fritch G. Evaluation of five methods for respiratory syncytial virus detection. *J Clin Microbiol* 1990; **28**: 1021–1025.
12. Freymuth F, Brooks W, Petitjean J, Daon F, Constantini S. Evaluation of an enzyme membrane immunoassay (Directigen RSV) for the rapid diagnosis of respiratory syncytial virus infections. *Pathol Biol* 1991; **39**: 283–286.
13. Pedneault L, Robillard L, Turgeon JP. Validation of respiratory syncytial virus enzyme immunoassay and shell vial assay results. *J Clin Microbiol* 1994; **32**: 2861–2864.
14. Reina J, Ros MJ, del Valle JM, Blanco I, Munar M. Evaluation of direct immunofluorescence, dot-blot enzyme immunoassay and shell-vial culture for detection of respiratory syncytial virus in patients with bronchiolitis. *Eur J Clin Microbiol Infect Dis* 1995; **14**: 1018–1020.
15. Dominguez EA, Taber LH, Couch B. Comparison of rapid diagnostic techniques for respiratory syncytial and influenza A virus respiratory infections in young children. *J Clin Microbiol* 1993; **31**: 2286–2290.
16. Englund JA, Piedra PA, Jewell A, Patel K, Baxter BB, Whimbey E. Rapid diagnosis of respiratory syncytial virus infections in immunocompromised adults. *J Clin Microbiol* 1996; **34**: 1649–1653.
17. Johnston SLG, Siegel CS. Evaluation of direct immunofluorescence, enzyme immunoassay, centrifugation culture, and conventional culture for the detection of respiratory syncytial virus. *J Clin Microbiol* 1990; **28**: 2394–2397.
18. Mendoza J, Navarro JM, Rojas A, de la Rosa M. Evaluation of immunofluorescence, two enzyme immunoassays and the shell-vial assay for detection of respiratory syncytial virus. *Eur J Clin Microbiol Infect Dis* 1991; **10**: 40–42.
19. Lipson SM, Popiolek D, Falk QZ, Bornfreund M, Krilow LR. Efficacy of Directigen RSV testing in patient management following admission from a paedriatric emergency department. *J Hosp Infect* 1999; **4**: 323–329.
20. Ribes JA, Seabolt JP, Overman SB. Performance characteristics of VIDAS and Directigen respiratory syncytial virus (RSV) antigen detection assays and culture for the identification of RSV in respiratory specimens. *J Clin Microbiol* 2002; **40**: 1818–1820.